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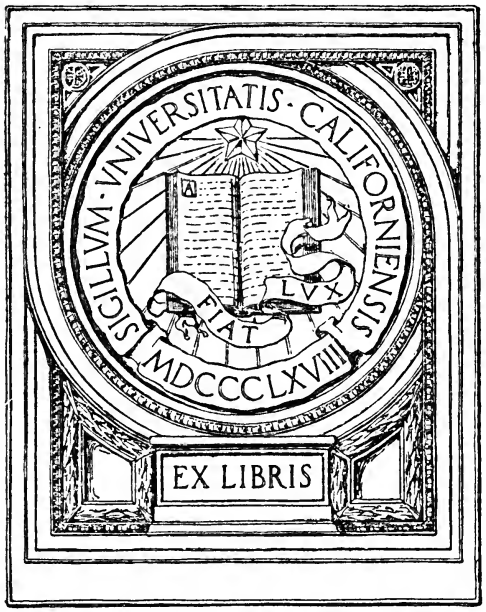

PRIZE ESSAY.

ON THE

CHEMICAL CONSTITUTION

OF

THE BILE.

BY

EDWARD R. TAYLOR, M. D.

CALIFORNIA.

9845

Divide et Impera.

EXTRACTED FROM THE

TRANSACTIONS OF THE AMERICAN MEDICAL ASSOCIATION.

PHILADELPHIA:

COLLINS, PRINTER, 705 JAYNE STREET.

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Regards of
Edward R. Taylor

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"'Tis hard, I confess, yet, nevertheless, I will adventure through the midst of these perplexities, and led by the clue or thread of the best writers, extricate myself out of a labyrinth of doubts and errors."

BURTON'S *Anatomy of Melancholy*.

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UNIV. OF
CALIFORNIA

PRIZE ESSAY.

THE CHEMICAL CONSTITUTION OF THE BILE.

THE bile is the most complex of all the juices, whether we consider its chemical nature or the purposes it subserves in the animal economy. The former has been more satisfactorily investigated by Strecker than by any other physiological chemist, his discovery, in 1848, of the resinous salts being beyond question a great step in advance. Much, however, remains yet to be done in this direction, for while we can form some idea as to how and where urea, creatine, creatinine, and the like, are formed, we are almost in the dark as to the tauro-cholic and glyco-cholic acids. The physiology of the bile is more obscure even than its chemistry. Indeed, we really *know* but little about it, and we therefore still sail upon the sea of conjecture.

Bile, as it is obtained from the gall-bladder, is a thick, ropy, viscid fluid, varying in specific gravity and color in different animals, the former being in man about 1018, and the latter being dark golden-brown according to some, and greenish-yellow according to others. Dalton fixes the specific gravity of human bile at 1018, ox bile at 1024, and pig's bile at 1030 to 1036. In chemical reaction it is found to be sometimes neutral, sometimes alkaline, but very rarely acid. It possesses antiseptic properties, and to this is attributed the fetor of the feces in some diseases, especially cholera, in which, owing to a general cessation of the secretions, the bile does not flow into the intestine. Its taste is excessively disagreeable and quite bitter, the after-taste being sweetish; and its odor peculiarly nauseous, being likened by Lehmann, especially when warmed, to that of musk. Owing to the contained mucus, it is susceptible of being drawn into threads (*Lehmann*); and it "froths up into a soap-like foam when shaken in a test-tube, or when air

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is forcibly blown into it through a small glass tube or blowpipe; the bubbles of foam thus produced remain for a long time without breaking, and adhere closely to each other and to the sides of the glass vessel" (*Dalton*). It is stated by Todd and Bowman, that by the addition of bile to the blood, coagulation is either retarded or prevented. This is accounted for on the ground of its affording a "mechanical obstacle" to the "cohesion of the particles of fibrin." It has been said that bile undergoes rapid decomposition when out of the body, but Pavy denies this, and asserts that such is the case only with weak or poor bile; and that rich bile dries up on exposure to the atmosphere, without showing signs of decomposition. The following analysis of the composition of the bile of the ox is from Dalton's physiology:—

Water	880.00
Glyco-cholate of soda Tauro-cholate of soda	90.00
Biliverdin (coloring matter) Fats Oleates, margarates, and stearates of soda, and potassa Cholesterine	13.42
Chloride of sodium Phosphate of soda " " lime " " magnesia Carbonates of soda and potassa	15.24
Mucus of gall-bladder	1.34
	1000.00

"Urea occurs in the bile in uræmia," and "albumen is found in the embryonic state, occasionally in fatty liver, in Bright's disease, and in abscess of the liver" (*Lehmann*).

Dr. Kemp gives the following analysis of the elementary proportions in the compound resulting from the union of the biliary organic principles with soda (choleic acid of Demarcay):—

Carbon	59.90
Hydrogen	8.90
Nitrogen	3.40
Oxygen	17.63
Sulphur	3.10
Soda	6.53
Chloride of sodium	0.54
	100.00

(*Budd on the Liver.*)

The following percentages of the solid constituents of the bile of different animals are taken from West's Dictionary of Chemistry: "In normal human bile, Frerichs found 14 per cent., or rather more, of solid constituents; Gorup-Besanez, in the bile of two recently executed criminals, found 10.19 and 17.73 per cent. solid matter; in that of an old man, 9.13 per cent.; and in that of a boy twelve years old, 17.19 per cent. Ox bile contains 10.13 per cent. solid constituents; pig's bile, 10.6 to 10.18 per cent. (*Gorup-Besanez and Strecker*); dog's bile, 5.1 per cent.; cat's bile, 5.6 per cent. (*Bidder and Schmidt*); sheep's bile, 5.13; rabbit's bile, 1.8; goose bile, 6.9; kangaroo's bile, 14.13; and crow's bile, 7.3 per cent. solid constituents." According to this, human bile is considerably richer in solid constituents than that of many animals, and somewhat richer than that of the ox and pig. This latter is not quite reconcilable with the specific gravities above given, for they would seem to show the reverse to be the case.

The fats, soaps, and inorganic salts of the bile require no mention, though, as regards the latter, it is a curious and important fact that in the bile of salt-water fishes the potassa salts predominate, while in that of fresh-water fishes the soda salts predominate. This was first noticed by Bensch, but was more fully investigated by Strecker (*Draper and Lehmann*).

Cholepyrrhine, the name given by Berzelius to the coloring matter of the bile, is a highly carbonaceous, uncrystallizable substance, containing a small quantity of nitrogen and iron; but its chemistry has, as yet, been so unsatisfactorily investigated, that little is known of its composition or properties. According to Lehmann, the original pigment is cholepyrrhine, a brownish substance, the biliverdin of Berzelius being the result of its oxidation. Berzelius thought that the bile pigment was composed of two coloring matters, on account of the precipitation of biliverdin from a soda

or potassa solution of cholepyrrhine, on the addition of an acid, but the view of Lehmann is now thought to be correct; and the various colors presented by bile are all probably dependent upon modifications of cholepyrrhine (*Kirke*). After its entrance into the intestine, the bile pigment is changed into a yellowish matter, which is so different from the original pigment, that nitric acid no longer brings out the play of colors so peculiar to the coloring matter of the bile. It is easily decomposed, so much so that it is extremely difficult to obtain it pure; it is sparingly soluble in most fluids, its best solvent being a solution of soda or potassa (*Todd and Bowman*). In jaundice, we find the bile pigment in various secretions, especially the urine, and in the blood. It forms a large proportion of biliary concretions, being generally associated in these with lime and cholesterine. From the researches of Bramson, it would seem that it forms with the former of these a definite compound. Its *origin* has been pretty well made out to be from the hæmatin of the blood-cell. Lehmann says that when the bile is long retained in the gall-bladder, there is produced a substance precisely similar to hæmatoidine; and Virchow, in his cellular pathology, remarks that hæmatoidine is the only substance in the body with which we are acquainted, that is allied to the bile pigment. "By the direct action of mineral acids, or after previous treatment and preparation by means of alkalies, the same, or precisely similar color tests, are obtained, which are yielded by the coloring matter of the bile, when treated with mineral acids; and it seems also, from other facts, that we have here a body before us which is very intimately connected with the coloring matter of the bile." He further remarks that in the interior of extravasations there arises a "yellowish-red substance which may be designated as a newly-formed kind of biliary coloring matter." In a case of cancer of the head of the pancreas in which the common duct was obstructed, I observed that the bile in the gall-bladder was inspissated (owing, no doubt, to its long retention there), and in color was ruby-red. This hue resulted from a concentration of yellow (precisely as is seen in the case of the blood), this being readily shown by the experiment of diluting a small quantity of the inspissated bile. Frerichs contests the above views, and maintains that no one has succeeded in manufacturing bile pigment from the red coloring matter of the blood. On the contrary, he holds that the biliary acids are the source of the bile pigment, and that the jaundice incident to pyæmia, and to many of the fevers, is owing

to an incomplete metamorphosis of the bile in the blood, and that "thus a sufficient quantity of bile pigment remains in the blood to give rise to all the symptoms of jaundice." In fact, by the continued action of sulphuric acid upon glyco-cholic acid, he asserts that a substance is produced whose reactions are the same as those of cholepyrrhine. The like result is obtained with the resinous salts. He injected colorless solutions of bile into the veins of dogs, and while Pettenkofer's test failed to show the biliary acids, nitric acid brought out all the play of colors characteristic of bile pigment. Kühne, however, maintains that the biliary acids *do* constitute a part of the urine in these cases, and says that the reason of their apparent absence is because of the Pettenkofer test not being sufficiently accurate. He claims to have detected the acids by means of the more delicate test of Hoppe, and says that when the biliary acids or their salts are injected into the veins, they pass out, as a rule, unchanged. Should any bile pigment be found, he explains its presence by reason of the dissolution of blood-corpuscles by the biliary acids and the consequent setting free of hæmatin, from which latter is produced the biliary coloring matter. Frerichs, from certain experiments in his laboratory (as we learn from the translator's preface to his clinical treatise on the liver), "admits that there is an intimate relation between bile pigment and the coloring matter of the blood, and even thinks it probable that the former substance may be developed from the latter," but still holds to his former belief that the biliary acids are convertible into bile pigment. Murchison, in his clinical lectures on the liver, leans towards the views of Frerichs. Niemeyer, however, holds the views of Kühne to be well established, for he says in the seventh edition (1869) of his practical medicine (I quote from Humphrey's and Hackley's translation), that the biliary acids "possess to a peculiar degree the property of dissolving the red blood-corpuscles. By injecting weak solutions of them into the blood of animals, we may artificially induce the so-called hæmatogenous icterus (jaundice without reabsorption), as the liberated coloring matter of the blood is transformed into biliary coloring matter. * * * * * The views regarding the occurrence of jaundice without retention and reabsorption of bile have totally changed since the observations of Virchow, Kühne, and Hoppe-Leyler have shown that bile coloring matter may be formed from the free coloring matter of the blood without the action of the liver; and we may induce artificial jaundice in animals by injecting substances

that dissolve the blood-corpuscles. There is now no doubt that some of the formerly enigmatical forms of icterus are due to the disintegration of the freed coloring matter circulating in the blood, into bile coloring matter." Besides, the iron that both contain would point directly to a close kinship between them. It would seem, therefore, that we may finally rest upon the belief that the source of the cholepyrrhine of the bile is the hæmatin of the blood.

The resinous salts, as they are called, glyco-cholate and tauro-cholate of soda, are, beyond question, the most important of the biliary ingredients, and form, with cholesterine, the essential constituents of the bile. As will be seen by reference to the analysis above given, they constitute 75 per cent. of the biliary solids. These two salts, as chemically described, exist only in the bile of the ox, for, as has been well shown by Dalton, there are minor differences to be found in them in the bile of various species of animals. Their reactions are not the same with the acetate and subacetate of lead; in some, the glyco-cholate crystallizes much more slowly than it does in ox bile, while in others, in human bile for instance, it does not crystallize at all. These substances are obtained from the bile of the ox by first evaporating it to dryness, and then extracting with absolute alcohol the resinous salts in conjunction with fats and coloring matter; the former are then obtained by treating the filtered solution with ether, which precipitates them, while the latter are dissolved. On standing for some little time in a test-tube, the glyco-cholate of soda is seen to shoot out in needle-like crystals, while the tauro-cholate remains in an amorphous condition somewhat resembling the globules of oil. They can be separated from each other by reason of their different reactions with the acetate and subacetate of lead (*Dalton*). We collate from West's Dictionary of Chemistry that the tauro-cholate of soda preponderates largely over the glyco-cholate in man, while the reverse is the case in the ox and the pig; in almost all other animals the tauro-cholate has the preponderance.

The *glyco-cholate of soda* crystallizes from its solution in radiating bundles of fine, white silky needles (*Dalton*). The soda being displaced by sulphuric acid, we have the glyco-cholic acid (cholic of Strecker) set free. Its formula is given as $C_{52}H_{42}NO_{11}$. It crystallizes in needles, and has a bitterish-sweet taste (*Fowne*). When boiled in a solution of potassa, it separates into two substances, cholic acid (cholalic of Strecker) and glycine; and when boiled in concentrated sulphuric or muriatic acid, the glycine is still pro-

duced, but the acid loses one atom of water and becomes choloidic acid. Dalton, in opposition to most physiological chemists, does not look upon glyco-cholic acid as being a conjugated compound, but takes the ground that the cholic acid and glycine are merely new combinations of its elements produced by long boiling in contact with potassa and water. To this view there are serious objections.

The Pettenkofer test brings out precisely the same colors when applied to the cholic acid as it does when applied to the glyco-cholic or tauro-cholic, which could scarcely be the case were the latter not conjugated acids with the former as a principal adjunct. Besides, in the decomposition of hippuric acid we have as marked an example of the separation of glycine as we have in the conjugated acids of the bile, this acid, when heated with concentrated nitric or muriatic acid, separating into benzoic acid and glycine. This latter, however, is thought by Lehmann to be formed from fumaramide (amide of fumaric acid), the glycine not existing preformed in the hippuric acid. His opinion is founded upon the fact that anhydrous glycine ($C_4H_4NO_3$)+anhydrous benzoic acid ($C_{14}H_5O_3$), yields one atom more of each hydrogen and oxygen than anhydrous hippuric acid contains. Those who take the ground that the glycine exists preformed (and the weight of opinion seems to preponderate this way), give to the hippuric acid an equivalent of water, making their formula, $C_{18}H_8NO_5HO$. The glycine, resulting from the decomposition of this acid, is the same as produced by the decomposition of glyco-cholic acid, and may be obtained artificially by boiling gelatine with a strong solution of potassa, which yields at the same time a substance called leucine. This might lead one to point to the metamorphosis of the gelatinous (connective) tissues as the source of glycine in the animal body; but, as Lehmann well remarks, the metamorphosis going on in these tissues is entirely too insignificant to account for the amount of glycine (or fumaramide) contained in the hippuric or glyco-cholic acids. He, therefore, refers its origin to the regressive metamorphosis of effete nitrogenous tissues, being analogous in this respect to urea. To be sure, the latter has been found in the blood, while nothing resembling glycine has been there discovered; but we must have consideration for the many difficulties accompanying the procurement from the blood of excrementitious substances, as well as for the fact that there is a large amount of uninvestigated matters put down as extractive, a knowledge of which will one day throw light on many places that are now dark. These extractives,

according to Lehmann's analysis, amount to as much as 2.60 parts in 1000 in the corpuscles, and 3.94 parts in 1000 in the liquor sanguinis. Pig's bile differs from that of all other animals examined, in that the glyco-cholic acid is replaced by an acid analogous to it, and known as hyocholic. This latter differs from the glyco-cholic in having two atoms more of carbon, one more of hydrogen, and one less of oxygen. It has never been found elsewhere than in the bile of the pig (*Lehmann*).

The *Tauro-Cholate of Soda* is an uncrystallizable substance, though Lehmann claims to have crystallized it. As has been mentioned, it exists in larger quantity in most animals than the glyco-cholate. The tauro-cholic acid (choleic of Strecker and bilin of other chemists) is best obtained by decomposing the tauro-cholate of soda with subacetate of lead, and treating the tauro-cholate of lead, thus produced, with sulphide of hydrogen (*Fowne*). It is said, however, never to have been obtained in a perfectly pure state. Its formula is given as $C_{52}H\ NS_2O_{14}$. It does not crystallize, is more soluble in water than glyco-cholic acid, and does not possess such strong acid properties. It dissolves the fatty acids, fats, and cholesterine, which will account for these substances being held in solution in the bile. Boiled with the alkalies, or mineral acids, it exhibits the same reactions as glyco-cholic acid, with the exception that taurine instead of glycine is developed; and the same remarks will apply to its conjugation as were made respecting that acid.

The soda, with which the glyco-cholic and tauro-cholic acids are united in the bile, is believed to be derived almost altogether from the chloride of sodium of the ingesta. This is decomposed in the stomach, the chlorine uniting with the hydrogen of water, and forming hydrochloric acid (the essential acid of digestion), and the sodium laying hold of the oxygen, and furnishing the soda for the bile.

Granted that the glyco-cholic and tauro-cholic acids are conjugated compounds, we are brought to the consideration of the glycine, taurine, and cholic acid, the most important inquiries being as to their source and mode of formation. Indeed, to succeed in this would be to answer the vital question relating to the chemistry of the bile. Before entering upon this, perhaps it would be well to make a brief examination of some of the physical properties of these interesting substances. Lehmann's great work on physiological chemistry being mainly relied upon as authority.

Glycine, or sugar of gelatine, or glycocoll ($C_4H_4NO_3$), is a ni-

trogenous substance, as its formula indicates, and has a sweetish taste, but no odor. It readily dissolves in cold water, but is almost insoluble in absolute alcohol, and quite so in ether. It is easily decomposed by electrolysis, there being an alkaline reaction at the negative, and an acid reaction at the positive pole. This inclined Horsford to the opinion that it was a salt-like compound with ammonia as a base, but Strecker puts it down as the amide of glycic acid, which Lehmann thinks to be correct. As stated above, it may easily be obtained from gelatine, from hippuric and glycocholic acids. According to Lehmann, it does not exist preformed in these acids, but is produced pretty much in the same manner as is glycerine from the oxide of lipyl, which, in the process of saponification, takes up an atom of water immediately on being set free, and becomes the hydrated oxide of lipyl, or glycerine.

Taurine ($C_4H_7NS_2O_6$) is a crystallizable, hard substance, characterized by its containing the sulphur which is found in the bile, as much as 25 per cent. of the taurine being made up of this element. When burned in the open air, it evolves sulphurous acid. It has a cooling taste, but no odor, and its solubility is about the same as that of glycine. Redtenbacher thought it to be a combination of sulphurous acid with aldehyde and ammonia, and attempted to produce it artificially by passing the acid into an alcoholic solution of aldehyde ammonia; but though he failed, a substance was obtained by him isomeric with, and very much resembling taurine. With the isethionate of ammonia, Strecker was more successful. By driving off its water, and treating the residue with alcohol, crystals of taurine were produced. This can be readily understood by examining the formulæ. Fowne gives that of isethionic acid as $C_4H_5O2SO_3$; add ammonia to this, and we have $C_4H_5O2SO_3NH_3$; or, to write it in another way, $C_4H_8O_7S_2N$. It is seen, therefore, that by the subtraction of the elements of one atom of water, we have expressed the formula of taurine. As to the formation of isethionic acid, we learn from Gregory that "when anhydrous acid SO_3 acts on alcohol, or when olefiant gas is absorbed by that dry acid, there is formed a compound, $2SO_3 + C_4H_4$ in crystals, which, when put into cold water, produce ethionic acid; when this solution is heated, 2 equivalents of sulphuric acid and 1 of alcohol separate from one-half, and there is formed from the other half isethionic acid." Thus, from such familiars as sulphuric acid, alcohol, and ammonia has man been able to manufacture one of the most complex substances in the animal organism!

Taurine has not been found isolated in normal conditions, but has been so found in decomposed or morbid bile. The moist tests for sulphur failing to show it in the bile, it is presumed that this element exists in the taurine in an oxidized state; but the chemistry of this substance is still very obscure. In the bile it is conjugated with cholic acid, forming the tauro-cholic acid.

Cholic acid, cholalic acid of Strecker ($C_{48}H_{39}O_9HO$), called by Carpenter the "fundamental component of bile," is a crystallizable, hard substance, characterized by its containing no nitrogen, a small proportion of oxygen, and a very large proportion of carbon—as much as 70½ parts in 100 being made up of this element. It is soluble in sulphuric acid; but, unlike taurine and glycine, it is difficultly soluble in water, while it dissolves in alcohol and ether. When boiled for some time with hydrochloric acid, it loses an atom of water, and with it its power of crystallization, becoming choloidic (choloidinic) acid. If the boiling be longer continued, this acid is changed into dyslysin—so called from its difficult solubility. By the action of nitric acid cholic acid is converted into capric, caprylic, and cholesteric acids (*Day*). Cholic acid is assumed by Lehmann to be a conjugated oleic acid, whose adjunct ($C_{12}H_6O_6$) is polymeric with cholesteric acid ($C_8H_4O_4$). Redtenbacher obtained this latter substance by the decomposition of choloidic acid. Having briefly passed in review some of the properties of glycine, taurine, and cholic acid, let us now inquire into something of what is known of their origin.

The afferent vessels of the liver are the portal vein and the hepatic artery—the latter a branch of the cœliac axis, while the former is made up of the superior and inferior mesenteric, the splenic and gastric veins, which receive the blood from the intestines, the stomach, and the spleen. It is probable, as is asserted by some (though denied by others), that the portal vein contains freshly digested matters from the stomach; and not only this, but fat from the small intestine. This latter has been established by the observations of Kölliker. He noticed that some hours after ingestion, sucking animals have invariably a fatty liver. "While of the same litter of animals some are made to fast, while others are allowed to suck, those which have sucked have a fatty liver a few hours afterwards, whilst the others have not" (*Virchow*). This fat could not come from the stomach, because we very well know that this organ does not act upon the fats at all, and that they are rendered absorbable elsewhere. It has also been pretty well

established, experimentally, that the veins have the predominance over the lacteals in the absorption of sugar from the small intestine. That the bile is not solely derived from the portal vein is proved by the experiment of its ligation, in which case the secretion still goes on, though in much diminished quantity. But, at the same time, this experiment, together with the fact that the hepatic artery does not have more than one-eighth the area of the portal vein, proves that the portal system is the chief source of the biliary secretion (*Carpenter*). Draper believes the bile to be derived altogether from systemic venous blood, and "not from the products of digestion obtained from the chylopoietic viscera." He thinks it inconceivable that products obtained from the digestive tract should be returned so soon thereto, and he cites the meconium of the fœtus as absolute proof; this being a true bile according to the analysis of Simon, and secreted before the system knows anything of digestion. But there can be nothing "inconceivable" in believing that freshly digested matters may be returned into the intestine in a metamorphosed shape, for the purpose of assisting in the digestion of other matters, or for the ulterior purpose of performing other service in the economy. And as to the meconium, it is not at all probable that the biliary secretion in the fœtus is the same as it becomes after birth. But, at the same time, one thing is certain: it is no proof that the constituents of bile do not pre-exist in the blood because they have not been found there. Our chemico-analytical investigations are beset with difficulties that science has as yet been unable to overcome; and we should further take into consideration the possible small quantity of bile as compared with that of the circulating blood. Liebig, in his Animal Chemistry, remarks, on this point, that "if we suppose $8\frac{1}{4}$ pounds (58,000 gr.) of blood to pass through the liver every minute, and if, from this quantity of blood, 2 drops of bile (3 gr. to the drop) are secreted, this would amount to $\frac{1}{9\,6\,0\,0}$th part of the weight of the blood—a proportion far too small to be quantitatively ascertained by analysis."

The cholic acid of the bile is held by Lehmann to be manufactured in the liver from fat and sugar. The former gives up its oleic acid, and the latter, on conjugation, is assumed to part with 6 atoms of water. We have seen that the portal blood is well supplied with these substances; and when we take into consideration the fact that olein, with proper care, gives the same reaction with Pettenkofer's test as cholic acid; that the hepatic veins are poorer

in oily, and richer in solid fat, than the portal vein; and that Redtenbacher obtained from choloidic acid (which, as has been mentioned, is cholic acid less one atom of water), when treated with nitric acid, the same volatile acids that, under similar circumstances, were obtained from oleine; the view of Lehmann seems very reasonable, and has much to support it. Schmidt, however, has a modification of it which is very ingenious. He derives both cholic acid and sugar from neutral fat, by replacing *one-seventh* of the hydrogen of glycerine with one atom of oxygen, which gives the formula for anhydrous grape sugar; and by assuming that *seven* of the equivalents of hydrogen of the solid fatty acid are replaced by oxygen, which gives the formula for cholic acid (*Lehmann*). Now, as an excess of fat and a deficiency of sugar enter the liver, while these conditions are reversed as they leave it, and as we know that the liver produces sugar in large quantity, Schmidt's view is certainly not unreasonable. Lehmann gives his opinion the preference over that of Schmidt, for these reasons: "Unconjugated acids containing nine atoms of oxygen are, at all events, very rare in chemistry; oleic acid yields the ordinary reaction with Pettenkofer's test, which is not the case with the solid fatty acids; and (what is of more importance) there is far less oily fat (although relatively more solid fat) in the hepatic venous blood than in that of the portal vein." Day, in his Physiological Chemistry, agrees with Lehmann as to cholic acid being formed from oleic acid and sugar, and remarks, that "a comparison of the respective products of oxidation of cholic and oleic acids strengthens the hypothesis; for when treated with concentrated nitric acid, cholic acid yields precisely the same products of decomposition as oleic acid, and additionally a carbo-hydrate, namely, cholesteric acid, whose composition is represented by the formula $C_8H_4O_4$." So far as the herbivora are concerned, Liebig thinks he has proved that the non-azotized products of digestion assist in the formation of the bile. He endeavors to show that the carbon of the bile, in these animals, is in larger amount than if it preserved its proper proportions to nitrogen as one of the constituents in the metamorphosis of nitrogenous tissue, and though there is much doubt as to the ox secreting so large an amount of bile in twenty-four hours as thirty-seven pounds, still the secretion is probably not sufficiently small to overthrow Liebig's calculation. The elements of starch furnish, he thinks, the non-nitrogenous constituents of bile, and he shows that by the separation of oxygen and the elements of water, cholo-

idic acid may be formed from starch. For instance, if from six atoms of starch we take forty-four atoms of oxygen and four atoms of water, we have remaining the formula of choloidic acid (*Liebig's Animal Chemistry*). That his views on this point are essentially the same as those of Lehmann, it is only necessary to quote a short extract from him: "Chemical analysis and the study of the living animal body mutually support each other; and both lead to the conclusion that a certain portion of the carbon of the non-azotized constituents of food (of starch, etc., the elements of respiration) is secreted by the liver in the form of bile." All this agrees with what has been before stated as to the portal vein containing freshly digested matters from the chylopoietic viscera.

As to the taurine and glycine, physiological chemists are well agreed that they are formed from the regressive metamorphosis of nitrogenous tissue, but the how and the where remain yet to be determined. The most careful manipulation has failed to detect their presence, or anything analogous to them, or any substance containing them, in the blood of the portal vein. Still, for reasons heretofore mentioned, there is a possibility of their having an existence there. We know that when the liver is unable to perform its functions, nitrogenous substances, possessing higher atomic numbers than glycine and taurine, are found abundantly in the liver and urine. In acute yellow atrophy, and in other cases of acholia, large quantities of leucine and tyrosine have been so discovered (*Frerichs*). Now, this fact alone would point to the liver as the seat of formation of glycine and taurine, and to the almost absolute certainty of their being formed from the regressive metamorphosis of nitrogenous tissue. It has been thought that the sulphuric acid of the portal blood gives origin to the sulphur of taurine; but while some chemists have found more of this acid in the portal blood than in that of the hepatic veins, other chemists, Lehmann for one, have found the reverse. This latter chemist has, however, found considerably more *sulphur* in the portal blood than in that of the hepatic veins, and he succeeded in obtaining an extract from the former which was very rich in this element. Still, he inclines to the opinion that the greater part of the sulphur of the bile is derived from the "perfect disintegration of fibrin in the liver." The blood undoubtedly undergoes great changes in its passage through this gland, among them being its loss of albumen and fibrin, and its proportionate gain of corpuscles. The former,

Lehmann thinks, builds up the walls of the latter (they in the blood of the hepatic veins containing no sulphur), while the fibrin is disintegrated and contributes to the formation of the bile. This is in accordance with what is pretty well established as to albumen being the parent of all the tissues, and with what is believed by many, that fibrin is the first product of the metamorphosis of albumen. In the liver, the fibrin, already on the downward course, may be decomposed into other regressive products that are worked up into the bile. We know that fibrin, when boiled in hydrochloric acid, yields tyrosine and lucine, and, as has been mentioned, that these substances accumulate in the liver and urine when the functions of the liver are arrested. Again, as is believed by Draper and others, the glycine and taurine may be formed in the ultimate tissues, as are urea, creatine, and the like, and be conveyed to the liver as are the latter to the kidneys, escaping detection in the blood. Liebig has attempted by paper formulæ to show how various excrementitious substances can be produced by the metamorphosis of other substances, and by the addition or subtraction of assumed quantities of water or oxygen, or both; but the whole matter rests upon his own assumptions, and simply shows that he is as capable in ingenuity as he is in chemical research. Some of his formulæ are, however, very plausible; and when we take into consideration the fact of his assumed quantities being taken alone from those substances (oxygen and water) that must play a great part in nearly every chemical metamorphosis which takes place in the body, we cannot but be led to think that he has, in these formulæ, opened up a road which will lead to many valuable truths.

There is yet to be considered one other constituent of the bile, *cholesterine*—a substance which, until quite recently, has been little investigated, but which, thanks to the labors of Flint, Jr., has become better known. His views I shall presently briefly state, giving them as I find them in a paper published by him in the *American Journal of Medical Sciences*, for October, 1862, entitled "New Excretory Function of the Liver."

Cholesterine—whose chemical constitution is variously put down, some making the formula $C_{25}H_{22}O$, some $C_{37}H_{32}O$, and others asserting still higher atomic numbers—is a crystallizable substance, soluble in ether and boiling alcohol, but sparingly soluble in cold alcohol, and insoluble in water. It is also freely soluble in the fatty

oils and in tauro-cholic acid, and hence is held in solution in the bile. Salisbury says it "is soluble in 9 parts of boiling alcohol of 0.84, and 5.55 parts of 0.816; in 12 parts of ether at 32° F., 3.7 at 59° F., and 2.2 at boiling; it is also soluble in wood-spirit, slightly soluble in boiling oil of turpentine and in water containing 4 parts of dry soap." Cholesterine is classed by Lehmann among the lipoids because of its strong resemblance to the fats, its difference from them being its incapability of saponification. It separates from its solution in layers of beautiful rhomboidal plates, whose extreme tenuity enables the observer to see the outlines of the lower plates through the substance of those that are superposed (*Dalton, and Flint, Jr.*). An aggregation of cholesterine crystals is one of the most beautiful of microscopic objects. Lehmann gives the angles of the tablets at 100° 30′ and 79° 30′, but Flint rejects the view of these angles being invariable, he having found them to be of different measurements; and from this circumstance, together with observations made during crystallization, he inclines to the opinion that the tablets are not regular crystals, but rather "fragments of micaceous sheets, which, from their extreme tenuity, are easily broken." Virchow has made out that the chemical reaction of cholesterine with sulphuric acid and iodine is the same as that of the cellulose of plants; iodine alone produces no change, but when the acid is added to the iodized mass, "its plates become colored, and assume, particularly at first, a brilliant indigo-blue tint, which gradually passes into a yellowish-brown, until the cholesterine is converted into a brownish drop." With concentrated sulphuric acid, cholesterine strikes a beautiful purple-red color (*Salisbury*). Lehmann states that cholesterine becomes electrical on friction. It has been found in many different situations, its most frequent habitat being the bile, blood, liver, meconium, brain, and nerves. The crystalline lens will also furnish it in large quantity. Flint denies its presence in normal feces, though others are said to have found it there. We find it in many pathological products; it forms a great part of biliary calculi; in fact, I recollect a case, in which in the gall-bladder of a woman there were found seventy two gall-stones, which were made up of pure cholesterine; in cancer, encysted tumors, the fluid of hydrocele and of ovarian cysts, tubercle, epithelial tumors, pus, degenerated ovaries and testes, and in pulmonary expectoration, cholesterine has been found in more or less quantity (*Lehmann and Flint*). I have met with it in can-

cer, in the fluid of ovarian cysts, and in atheromatous matter. Lehmann says that he "once found the choroid plexus in the brain perfectly incrusted with cholesterine," and he considers it to be "an integral constituent of pus." I have known pieces of abnormal brain and spinal cord that had remained in alcohol for a short time to become quite brilliant from the numerous crystals of cholesterine that had formed on their surfaces. Virchow asserts it to be a constant ingredient of atheromatous matter, and further, that in "every case where fatty products remain stagnant for a considerable time within a closed cavity in which but little interchange of matter can go on, the fat sets free cholesterine" (*Cellular Pathology*). Salisbury (see April number, 1863, *American Journal of Medical Sciences*) has found cholesterine in numerous situations as follows: in the ova of the human subject and of animals; in the seminal fluid of man; in the saliva; in the effusion from congested or inflamed mucous surfaces; in the fluid of ascites; in the fluid of spina bifida tumors; in the tears; in the milk of the cow, and in that of woman before and soon after delivery; in butter, beef, and hog suet; in the secretion of the sudorific glands, and in the urine in jaundice, intermittent fever, varicella, diphtheria, diabetes mellitus, remittent and typhoid fevers. He agrees with Flint as to its being an effete product of the nervous system, but thinks his observations prove that the liver is not the only organ which separates it from the blood. The analyses of Flint show that cholesterine exists in much larger quantity in the blood than has been suspected. While Becquerel and Rodier obtained only .090 parts of cholesterine in 1000 parts of venous blood, Flint was able to obtain as much as .445, .751, and .658. He accounts for this great difference of results on the ground that while others operated merely on the serum, he operated on both the clot and serum.

Concerning the origin of cholesterine, there was nothing but conjecture until Flint's investigations. That this statement is true, it is only necessary to glance at what has been said on this subject by leading physiological chemists. And it seems that there is every reason to believe that Flint's investigations in this direction establish as truthful his proposition that "cholesterine is an excrementitious product formed in great part by the destructive assimilation of the brain and nerves." It will be interesting to briefly glance at some of the proofs by which he seeks to establish this proposition. In the first instance, he took some dog's blood from

the internal jugular, carotid, vena cava, hepatic veins, hepatic artery and portal vein, in the order in which they are named, and extracted the cholesterine from each. This experiment demonstrated to his satisfaction that there was a much larger quantity of cholesterine in the veins returning the blood *from* the brain, the pelvic organs, and the lower extremities, than there was in the vessels carrying the blood *to* those organs. Now, as it is known that the *nervous tissue* is the only one that normally furnishes cholesterine in any quantity, it should seem that the blood, in passing through it, must acquire the cholesterine from that tissue. But Flint was not satisfied until he had made a quantitative analysis. For this purpose, he made three experiments on dogs, taking blood from the carotid, internal jugular, and femoral vein of two of them, and from one of them blood from the two former. The first experiment demonstrated that there was an increase of cholesterine in the blood of the jugular vein over the arterial blood of 3.488 per cent., and an increase in the femoral vein of 4.134 per cent. Thinking that the etherization might affect the nutrition of the nervous tissue, especially that of the brain, he operated, in the next two experiments, without administering an anæsthetic. In this he was correct, for both experiments demonstrated a much greater increase of cholesterine in the venous blood over the arterial blood than did the first experiment. In one case the increase in the blood of the jugular vein was 59.772, and in that of the femoral vein, 6.308 per cent. In the last experiment of this series, the increase in the blood of the jugular was 23.307 per cent. I merely sketch here what, in Flint's paper, is drawn out in admirable detail; but sufficient has been put down to reasonably prove that cholesterine is acquired by the blood in its passage through the nervous tissue. There is still other proof, however. If cholesterine be the product of the destructive assimilation of nervous tissue, it should be produced in proportion to the activity of that tissue; and in cases of paralysis, where the nutritive forces in the parts affected are so much interfered with that the tissue sometimes becomes disorganized, we should have a diminution in the quantity of cholesterine of the venous blood. This was found to be the case. Flint operated on hemiplegic patients by taking blood from both arms, and analyzing it for cholesterine. It is well worth while to subjoin the table of his results:—

	Blood. Grains.	Cholesterine. Grains.	Cholesterine per 1000.
Case I.			
Paralyzed side....	55.458	.....	The watch-glass contained 0.031 grains of a substance, but the most careful examination failed to show a single crystal of cholesterine.
Sound side.....	128.407	0.062	0.481.
Case II.			
Paralyzed side....	18.381	.....	Same as Case I.
Sound side.....	66.396	0.062	0.808.
Case III.			
Paralyzed side....	21.842	.....	Same as Case I.
Sound side.....	52.261	0.031	0.579.

Flint reasserts his views in regard to cholesterine in his recent work on physiology, and they would seem to rest upon a tolerably secure foundation. In cases of acholia, the unconsciousness, delirium, and coma (the cause of which has puzzled all the observers) are attributed by him to the excess of cholesterine in the blood, producing a condition which has been called cholesteremia, in analogy to uremia.

Cholesterine, then, as the above series of experiments seem to prove, is the product of the destructive assimilation of nervous tissue and is carried by the blood to the liver, where it becomes a constituent of the bile. In the former it is thought to be held in solution by the fatty acids, while in the latter the biliary organic salts are the agents which render it soluble. As regards this substance, it is interesting to cite the fact that it is the only one of the constituents of the bile proper that has been found either in the blood or any of the tissues. And though this by no means proves that its fellows are formed in the liver, still it should have some weight in our considerations concerning their origin, and when our attention is drawn to analogies between the kidney and the liver, we must keep in mind the fact that *every organic constituent of the urine* has been found in certain tissues and some of them in the blood; while, as yet, but *one organic constituent of the bile* has been so found. We have seen that the respective constitutions of the blood of the portal and hepatic veins will account for the origin of the bile; still, as our blood analyses are not yet perfect, our present knowledge on this subject can be deemed little more than reasonable conjecture.

Tests for Bile.—The peculiar and striking play of colors produced by treating bile with nitric acid has been long well known,

the colors passing through the shades of blue, violet, red, and green. The acid should be applied in small quantity to a very thin layer of the suspected liquid if we wish to see the colors in all their beauty. If the acid be added to a mixture containing bile, and the mixture be shaken up, a dense precipitate, grass-green in color, will be thrown down; and even on exposure to the air, in an open glass vessel, for a few hours, the surface will gradually assume a greenish tinge, which length of time deepens (*Dalton*). If muriatic acid be added to the colored liquid, and the mixture be boiled for a short time, the green color will be still more strikingly brought out (*Brande and Taylor's Chemistry*). The green pigment thus produced has been called biliverdin, and is said to have the same properties as chlorophyll, the coloring matter of plants. It is highly probable that the nitric acid operates by oxidizing the cholepyrrhin, as we know it does in the act of cauterization, or of dissolving those metals that are unable to resist its power. Nature furnishes us with analogies. The beautifully diversified hues that the leaves of the forest assume in the "melancholy days" of autumn, are the product of the slow oxidation of chlorophyll; the vitality of the leaf, kept vigorous by the breath of summer, being weakened as the chilling breezes prevail, is compelled to submit to the play of chemical forces.[1] The hæmatin preserves its color when circulating with the blood in the vessels of the body; but when the blood is extravasated, its pigment is overcome by the chemical forces, and presents all the colors of the rainbow. Nitric acid, however, is no test for bile—it simply reveals the presence of its coloring principle; and if the latter were absent, no colors would be produced. Some other test, therefore, is necessary, and the readiest that is known to chemistry is that of Pettenkofer. This consists essentially in the reaction that the bile, or its chief constituents (the resinous salts), gives with sulphuric acid and cane sugar. A drop of syrup is added to about half an ounce of the suspected liquid, and the acid then added until a red color is made manifest; this

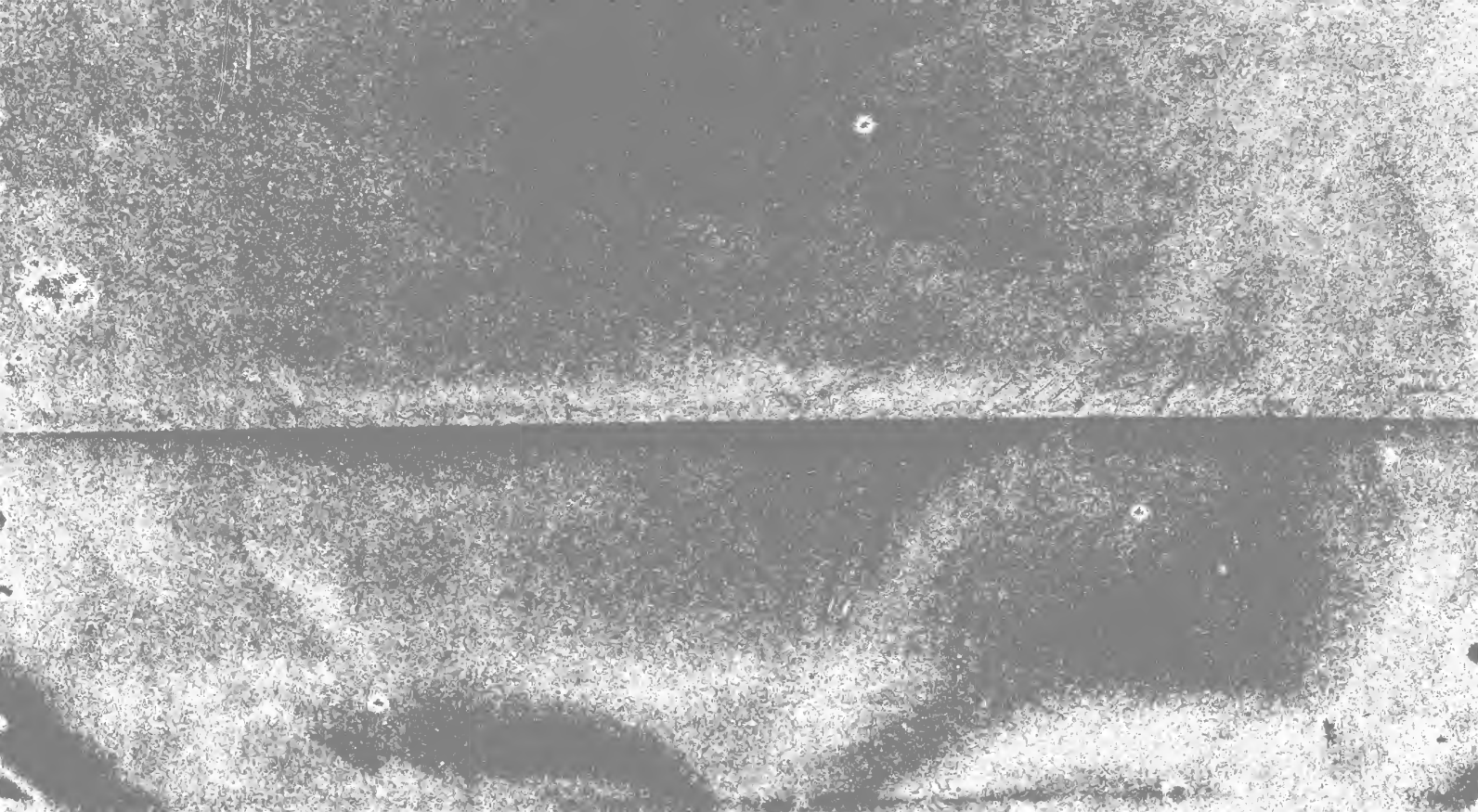
[1] I would not wish to be understood as being firmly fixed in the belief that *vital* effects are not, or cannot, be produced by *physical* causes; or that the vital principle is a distinct entity which stands guard over the tissues and controls their action; chemistry has produced urea, glycine, taurine, and other organic compounds outside of the body, and it is hence reasonable to presume that they are thus produced on the inside of it. There may be a special vital force, distinct from the other forces of which we are cognizant, and independent of the control of physical law; but this is an hypothesis, which, so far from being verified, seems to be seriously endangered by the advance of science.

slowly passes into a lake, and ultimately into a purple. If the quantity of bile is very small, and the reaction cannot well be made out, it will be necessary to separate the resinous salts from the mixture. This can be done by first evaporating to dryness, and then treating the residue with boiling alcohol; the resinous salts are thus dissolved out, together with the coloring matter, cholesterine, and more or less of the fats. The alcohol is then evaporated, and the residue treated with a small quantity of ether; the precipitate at first produced will disappear on the mixture being shaken, but when the ether is added in excess, the precipitate remains permanent, and consists of the tauro-cholate and glycocholate of soda (*Dalton and Fowne*). These latter must be dissolved in water before the test is applied. The sugar must be used in very small quantity, and the acid cautiously and gradually added, the first change of color being intently looked for. As soon as the first tints of red appear, the addition of the acid must be stopped (*Dalton*). But it is not sufficient to indicate the presence of the resinous salts when we produce the red reaction. Unless this color pass successively into lake and purple, no bile is before us; and it is the "lake and purple color alone which can be regarded as really characteristic of the biliary reaction" (*Dalton*). As has been stated before, olein is susceptible to the Pettenkofer test; but the colors are much longer in being developed, and, as Lehmann remarks, take place in their layers, as, for instance, on a watch-glass. Oil of turpentine and of caraway are likewise susceptible, but the red color becomes brown and blackish, and a tarry, empyreumatic odor is produced. Besides, none of these substances are likely to be found in the animal juices; and, moreover, we have the unfailing resource of extracting the organic salts from the suspected liquid, if they be present.

Hoppe's test, of which mention has been made, is more delicate than Pettenkofer's, but very complicated, and is as follows:—

"1. Decompose the icteric urine to be examined with an excess of milk of lime; 2. Boil for about half an hour; 3. Filter; 4. Evaporate the filtered fluid nearly to dryness; 5. Decompose with a great excess of concentrated hydrochloric acid, and then keep the whole (before being again filtered) at the boiling point for half an hour; 6. To avoid spurting of the fluid, it is necessary to renew the volatilized hydrochloric acid from time to time; 7. Leave the liquid to get completely cold, and then add six to eight times its volume of water; 8. Filter the dark brown turbid solu-

tion thus obtained, and wash out with water the residue on the filter, until the same runs through quite colorless; 9. Dissolve the brown resinous mass on the filter in 90 per cent. alcohol; 10. Decolorize by boiling with animal charcoal, filter, and evaporate to dryness in the water bath; the residue is a yellow, resinous mass, which, if bile acids be present, must consist, for the most part, of pure *choloidic acid.* In such a case it melts by warming, and emits the peculiar musk or soap odor. 11. Lastly, dissolve in a very little caustic soda and some drops of warm water, add a very small piece of sugar, and allow three drops of concentrated SO_3 slowly to fall into it. At first the fluid becomes milky and troubled, and resinous flakes separate, which stick pertinaciously to the glass, but afterwards, by the addition of more SO_3, these again dissolve, and produce a beautiful purple-red or dark violet fluid" (*Kühne* as quoted by *Aitken*). It is thus seen that Hoppe relies upon sulphuric acid and sugar the same as Pettenkofer, and that his method consists essentially in the procurement from the bile of choloidic acid upon which to use those reagents.



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